

**Amendments to the Claims:**

This listing replaces all prior versions and listings of claims in the application:

**Listing of Claims:**

1. (currently amended) A method for selecting ~~siRNA comprising selecting an a~~ rationally designed siRNA, wherein said rationally designed siRNA comprising ~~comprises selecting an siRNA molecule of 19 – 25 nucleoside bases~~ base pairs, said method comprising:
  - (a) selecting a target gene, wherein the target gene comprises a target sequence;
  - (b) ~~measuring the functionality of sequences of 19 – 25 nucleotides in length that~~ are substantially complementary to a stretch of nucleotides of the target ~~sequence, wherein said functionality is dependent upon non-target specific~~ criteria;
  - (b) applying at least one non-target specific criterion to at least one candidate siRNA sequence, wherein the at least one non-target specific criterion comprises the presence or absence of a particular nucleotide at at least one of sequence positions 1-19 of the siRNA, and the at least one candidate siRNA sequence comprises an antisense strand that is at least substantially complementary to a stretch of nucleotides of the target sequence; and
  - (c) selecting a rationally designed siRNA from said at least one candidate siRNA.
2. (currently amended) The method according to claim 1, wherein (b) comprises applying at least one of Formulas I, II, and IV-IX to said at least one candidate siRNA, wherein Formulas I-IX are ~~wherein said functionality is determined by applying one of~~ the following formulas:

$$\text{Formula I} = -(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C}) * 3 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) \\ + (U_{10}) + (A_{14}) - (U_5) - (A_{11});$$

$$\text{Formula II} = -(GC/3) - (AU_{15-19}) * 3 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3);$$

~~$$\text{Formula III} = -(GC/3) + (AU_{15-19}) - (Tm_{20^\circ C}) * 3;$$~~

$$\text{Formula IV} = -(GC/2) + (AU_{15-19})/2 - (Tm_{20^\circ C}) * 2 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) \\ + (U_{10}) + (A_{14}) - (U_5) - (A_{11});$$

$$\text{Formula V} = -(G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11});$$

$$\text{Formula VI} = -(G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3);$$

$$\text{Formula VII} = -(GC/2) + (AU_{15-19})/2 - (Tm_{20^\circ C}) * 1 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 3 \\ + (A_3) * 3 + (U_{10})/2 + (A_{14})/2 - (U_5)/2 - (A_{11})/2;$$

wherein in Formulas I, II, and IV – VII:

$AU_{15-19} = 0 - 5$  depending on the number of A or U bases on the sense strand at positions 15 –19;

$G_{13} = 1$  if G is the base at position 13 on the sense strand, otherwise its value is 0;

$C_{19} = 1$  if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC = the number of G and C bases in the entire sense strand;

$Tm_{20^\circ C} = 1$  if the  $Tm$  is greater than  $20^\circ C$ ;

$A_3 = 1$  if A is the base at position 3 on the sense strand, otherwise its value is 0;

$A_{11} = 1$  if A is the base at position 11 on the sense strand, otherwise its value is 0;

$A_{14} = 1$  if A is the base at position 14 on the sense strand, otherwise its

value is 0;

$A_{19} = 1$  if A is the base at position 19 on the sense strand, otherwise its value is 0;

$U_5 = 1$  if U is the base at position 5 on the sense strand, otherwise its value is 0;

$U_{10} = 1$  if U is the base at position 10 on the sense strand, otherwise its value is 0;

or

Formula VIII:  $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$ ; and

Formula IX:  $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-Tm-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein in Formula VIII and Formula IX

$A_1 = 1$  if A is the base at position 1 of the sense strand, otherwise its value is 0;

$A_2 = 1$  if A is the base at position 2 of the sense strand, otherwise its value is 0;

$A_3 = 1$  if A is the base at position 3 of the sense strand, otherwise its value is 0;

$A_4 = 1$  if A is the base at position 4 of the sense strand, otherwise its value is 0;

$A_5 = 1$  if A is the base at position 5 of the sense strand, otherwise its value is 0;

$A_6 = 1$  if A is the base at position 6 of the sense strand, otherwise its value is 0;

$A_7 = 1$  if A is the base at position 7 of the sense strand, otherwise its value is 0;

$A_{10} = 1$  if A is the base at position 10 of the sense strand, otherwise its value is 0;

$A_{11} = 1$  if A is the base at position 11 of the sense strand, otherwise its value is 0;

$A_{13} = 1$  if A is the base at position 13 of the sense strand, otherwise its value is 0;

$A_{19} = 1$  if A is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$C_3 = 1$  if C is the base at position 3 of the sense strand, otherwise its value is 0;

$C_4 = 1$  if C is the base at position 4 of the sense strand, otherwise its value is 0;

$C_5 = 1$  if C is the base at position 5 of the sense strand, otherwise its value is 0;

$C_6 = 1$  if C is the base at position 6 of the sense strand, otherwise its value is 0;

$C_7 = 1$  if C is the base at position 7 of the sense strand, otherwise its value is 0;

$C_9 = 1$  if C is the base at position 9 of the sense strand, otherwise its value is 0;

$C_{17} = 1$  if C is the base at position 17 of the sense strand, otherwise its value is 0;

$C_{18} = 1$  if C is the base at position 18 of the sense strand, otherwise its value is 0;

$C_{19} = 1$  if C is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$G_1 = 1$  if G is the base at position 1 on the sense strand, otherwise its value is 0;

$G_2 = 1$  if G is the base at position 2 of the sense strand, otherwise its value is 0;

$G_8 = 1$  if G is the base at position 8 on the sense strand, otherwise its value is 0;

$G_{10} = 1$  if G is the base at position 10 on the sense strand, otherwise its value is 0;

$G_{13} = 1$  if G is the base at position 13 on the sense strand, otherwise its value is 0;

$G_{19} = 1$  if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$U_1 = 1$  if U is the base at position 1 on the sense strand, otherwise its value is 0;

$U_2 = 1$  if U is the base at position 2 on the sense strand, otherwise its value is 0;

$U_3 = 1$  if U is the base at position 3 on the sense strand, otherwise its value is 0;

$U_4 = 1$  if U is the base at position 4 on the sense strand, otherwise its value is 0;

$U_7 = 1$  if U is the base at position 7 on the sense strand, otherwise its value is 0;

$U_9 = 1$  if U is the base at position 9 on the sense strand, otherwise its value is 0;

$U_{10} = 1$  if U is the base at position 10 on the sense strand, otherwise its value is 0;

$U_{15} = 1$  if U is the base at position 15 on the sense strand, otherwise its value is 0;

$U_{16} = 1$  if U is the base at position 16 on the sense strand, otherwise its value is 0;

$U_{17} = 1$  if U is the base at position 17 on the sense strand, otherwise its value is 0;

$U_{18} = 1$  if U is the base at position 18 on the sense strand, otherwise its value is 0;

$GC_{15-19}$  = the number of G and C bases within positions 15 – 19 of the sense strand or within positions 15 –18 if the sense strand is only 18 base pairs in length;

$GC_{total}$  = the number of G and C bases in the sense strand;

$T_m = 100$  if the targeting site contains an inverted repeat longer than 4 base pairs, otherwise its value is 0; and

X = the number of times that the same nucleotide repeats four or more times in a row.

3. (currently amended) A method of gene[[-]] silencing comprising selecting ~~an~~ said rationally designed siRNA according to claim 2 and introducing ~~it~~ said rationally designed siRNA into a cell.
4. (currently amended) The method according to claim 3 wherein said introducing is by allowing passive uptake of the rationally designed siRNA.
5. (original) The method according to claim 3, wherein said introducing is through the use of a vector.
6. (currently amended) A method for developing an siRNA algorithm for selecting a rationally designed siRNA, wherein said rationally designed siRNA is capable of silencing a target gene, said method comprising:
  - (a) selecting a set of siRNA, wherein the set of siRNA comprises a plurality of siRNA that are directed against sequences in the target gene;
  - (b) ~~measuring~~ determining the ~~gene silencing~~ ability of each siRNA ~~from~~ of said ~~set~~ plurality of siRNA to silence the target gene;
  - (c) ~~determining the amount of improved functionality by correlating the~~ presence or absence of at least one variable with said ability of each

siRNA to silence said target gene, wherein said at least one variable is selected from the group consisting of the total GC content, melting temperature of the siRNA, GC content at positions 15 –19, the presence or absence of a particular nucleotide at a particular position and the number of times that the same nucleotide repeats within a given sequence; and

- (d) developing an algorithm for selecting siRNA that are capable of silencing a target gene, wherein said algorithm comprises said at least one variable using the information of step (d).

7. (canceled)

8. (currently amended) A method of selecting hyperfunctional siRNA, said method comprising ~~using at least one functional siRNA, wherein at least one said functional siRNA has been selected according to the method of claim 7 and~~ measuring the silencing ability of said rationally designed said at least one functional siRNA of claim 1, wherein said silencing ability is measured at a concentration of less than 1 nanomolar siRNA, and selecting said rationally designed siRNA if said rationally designed siRNA either induces greater than 95% silencing or induces at least functional levels of silencing for greater than 96 hours.

9.-18. (canceled)

19. (currently amended) A kit for silencing a target gene, wherein said kit is ~~comprised of~~ comprises at least two siRNA, wherein said at least two siRNA comprise a first optimized siRNA and a second optimized siRNA, wherein said first optimized siRNA and said second optimized siRNA ~~are optimized~~ comprise sequences that are selected according to at least one of the following formulas:

Formula I =  $-(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C}) * 3 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$ ;

Formula II =  $-(GC/3) - (AU_{15-19}) * 3 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3)$ ;

Formula III =  $-(GC/3) + (AU_{15-19}) - (Tm_{20^{\circ}C}) * 3$ ;

Formula IV =  $-(GC/2) + (AU_{15-19})/2 - (Tm_{20^{\circ}C}) * 2 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$ ;

Formula V =  $-(G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3) + (U_{10}) + (A_{14}) - (U_5) - (A_{11})$ ;

Formula VI =  $-(G_{13}) * 3 - (C_{19}) + (A_{19}) * 2 + (A_3)$ ;

Formula VII =  $-(GC/2) + (AU_{15-19})/2 - (Tm_{20^{\circ}C}) * 1 - (G_{13}) * 3 - (C_{19}) + (A_{19}) * 3 + (A_3) * 3 + (U_{10})/2 + (A_{14})/2 - (U_5)/2 - (A_{11})/2$ ;

wherein in Formulas I – VII:

$AU_{15-19}$  = 0 – 5 depending on the number of A or U bases on the sense strand at positions 15 – 19;

$G_{13}$  = 1 if G is the base at position 13 on the sense strand, otherwise its value is 0;

$C_{19}$  = 1 if C is the base at position 19 of the sense strand, otherwise its value is 0;

GC = the number of G and C bases in the entire sense strand;

$Tm_{20^{\circ}C}$  = 1 if the  $Tm$  is greater than  $20^{\circ}C$ ;

$A_3$  = 1 if A is the base at position 3 on the sense strand, otherwise its value is 0;

$A_{11}$  = 1 if A is the base at position 11 on the sense strand, otherwise its

value is 0;

$A_{14} = 1$  if A is the base at position 14 on the sense strand, otherwise its value is 0;

$A_{19} = 1$  if A is the base at position 19 on the sense strand, otherwise its value is 0;

$U_5 = 1$  if U is the base at position 5 on the sense strand, otherwise its value is 0;

$U_{10} = 1$  if U is the base at position 10 on the sense strand, otherwise its value is 0;

or

Formula VIII:  $(-14)*G_{13}-13*A_1-12*U_7-11*U_2-10*A_{11}-10*U_4-10*C_3-10*C_5-10*C_6-9*A_{10}-9*U_9-9*C_{18}-8*G_{10}-7*U_1-7*U_{16}-7*C_{17}-7*C_{19}+7*U_{17}+8*A_2+8*A_4+8*A_5+8*C_4+9*G_8+10*A_7+10*U_{18}+11*A_{19}+11*C_9+15*G_1+18*A_3+19*U_{10}-T_m-3*(GC_{total})-6*(GC_{15-19})-30*X$ ; and

Formula IX:  $(14.1)*A_3+(14.9)*A_6+(17.6)*A_{13}+(24.7)*A_{19}+(14.2)*U_{10}+(10.5)*C_9+(23.9)*G_1+(16.3)*G_2+(-12.3)*A_{11}+(-19.3)*U_1+(-12.1)*U_2+(-11)*U_3+(-15.2)*U_{15}+(-11.3)*U_{16}+(-11.8)*C_3+(-17.4)*C_6+(-10.5)*C_7+(-13.7)*G_{13}+(-25.9)*G_{19}-T_m-3*(GC_{total})-6*(GC_{15-19})-30*X$

wherein in Formulas VIII and IX

$A_1 = 1$  if A is the base at position 1 of the sense strand, otherwise its value is 0;

$A_2 = 1$  if A is the base at position 2 of the sense strand, otherwise its value is 0;

$A_3 = 1$  if A is the base at position 3 of the sense strand, otherwise its value is 0;

$A_4 = 1$  if A is the base at position 4 of the sense strand, otherwise its value is 0;

$A_5 = 1$  if A is the base at position 5 of the sense strand, otherwise its value is 0;

$A_6 = 1$  if A is the base at position 6 of the sense strand, otherwise its value is 0;

$A_7 = 1$  if A is the base at position 7 of the sense strand, otherwise its value is 0;

$A_{10} = 1$  if A is the base at position 10 of the sense strand, otherwise its value is 0;



$A_{11} = 1$  if A is the base at position 11 of the sense strand, otherwise its value is 0;  
 $A_{13} = 1$  if A is the base at position 13 of the sense strand, otherwise its value is 0;  
 $A_{19} = 1$  if A is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$C_3 = 1$  if C is the base at position 3 of the sense strand, otherwise its value is 0;  
 $C_4 = 1$  if C is the base at position 4 of the sense strand, otherwise its value is 0;  
 $C_5 = 1$  if C is the base at position 5 of the sense strand, otherwise its value is 0;  
 $C_6 = 1$  if C is the base at position 6 of the sense strand, otherwise its value is 0;  
 $C_7 = 1$  if C is the base at position 7 of the sense strand, otherwise its value is 0;  
 $C_9 = 1$  if C is the base at position 9 of the sense strand, otherwise its value is 0;  
 $C_{17} = 1$  if C is the base at position 17 of the sense strand, otherwise its value is 0;  
 $C_{18} = 1$  if C is the base at position 18 of the sense strand, otherwise its value is 0;  
 $C_{19} = 1$  if C is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$G_1 = 1$  if G is the base at position 1 on the sense strand, otherwise its value is 0;  
 $G_2 = 1$  if G is the base at position 2 of the sense strand, otherwise its value is 0;  
 $G_8 = 1$  if G is the base at position 8 on the sense strand, otherwise its value is 0;  
 $G_{10} = 1$  if G is the base at position 10 on the sense strand, otherwise its value is 0;  
 $G_{13} = 1$  if G is the base at position 13 on the sense strand, otherwise its value is 0;  
 $G_{19} = 1$  if G is the base at position 19 of the sense strand, otherwise if another base is present or the sense strand is only 18 base pairs in length, its value is 0;

$U_1 = 1$  if U is the base at position 1 on the sense strand, otherwise its value is 0;  
 $U_2 = 1$  if U is the base at position 2 on the sense strand, otherwise its value is 0;  
 $U_3 = 1$  if U is the base at position 3 on the sense strand, otherwise its value is 0;  
 $U_4 = 1$  if U is the base at position 4 on the sense strand, otherwise its value is 0;  
 $U_7 = 1$  if U is the base at position 7 on the sense strand, otherwise its value is 0;  
 $U_9 = 1$  if U is the base at position 9 on the sense strand, otherwise its value is 0;

$U_{10} = 1$  if U is the base at position 10 on the sense strand, otherwise its value is 0;

$U_{15} = 1$  if U is the base at position 15 on the sense strand, otherwise its value is 0;

$U_{16} = 1$  if U is the base at position 16 on the sense strand, otherwise its value is 0;

$U_{17} = 1$  if U is the base at position 17 on the sense strand, otherwise its value is 0;

$U_{18} = 1$  if U is the base at position 18 on the sense strand, otherwise its value is 0;

$GC_{15-19}$  = the number of G and C bases within positions 15 – 19 of the sense strand or within positions 15 –18 if the sense strand is only 18 base pairs in length;

$GC_{total}$  = the number of G and C bases in the sense strand;

$T_m = 100$  if the targeting site contains an inverted repeat longer than 4 base pairs, otherwise its value is 0; and

$X$  = the number of times that the same nucleotide repeats four or more times in a row.

20. (new) The method according to claim 1, wherein the particular nucleotide is A and the sequence position is position 19.

21. (new) The method according to claim 1, wherein said at least one candidate siRNA comprises at least two candidate siRNAs.

22. (new) The method according to claim 21, wherein said at least two candidate siRNAs comprise at least three candidate siRNAs.

23. (new) The method according to claim 22, wherein said at least three candidate siRNAs comprise at least four siRNAs.

24. (new) The method according to claim 23, wherein said at least four candidate siRNAs comprise all potential siRNAs for said target gene that are 19 base pairs in length and 100% complementary to regions of said target gene.

25. (new) The method according to claim 1, wherein said antisense strand of said rationally designed siRNA is 100% complementary to said target sequence.
26. (new) The method according to claim 24, wherein said antisense strand of said rationally designed siRNA is 100% complementary to said target sequence.
27. (new) The method according to claim 6, wherein said at least one variable comprises at least two variables selected from the group consisting of: the total GC content, melting temperature of the siRNA, GC content at positions 15 -19, the presence or absence of one or more particular nucleotides at one or more particular positions, and the number of times that the same nucleotide repeats within a given sequence and said algorithm comprises said at least two variables.
28. (new) The method according to claim 27, wherein said at least two variables comprise at least three variables selected from the group consisting of: the total GC content, melting temperature of the siRNA, GC content at positions 15 -19, the presence or absence of one or more particular nucleotides at one or more particular positions, and the number of times that the same nucleotide repeats within a given sequence and said algorithm comprises said at least three variables.
29. (new) The method according to claim 28, wherein said at least three variables comprise at least four variables selected from the group consisting of: the total GC content, melting temperature of the siRNA, GC content at positions 15 -19, the presence or absence of one or more particular nucleotides at one or more particular positions, and the number of times that the same nucleotide repeats within a given sequence and said algorithm comprises said at least four variables.
30. (new) The method according to claim 1, wherein the at least one non-target specific criterion comprises the presence or absence of particular nucleotides at at least two of

sequence positions 1 - 19 of the at least one candidate siRNA, wherein said particular nucleotides may be the same or different nucleotides.

31. (new) The method according to claim 1, wherein the at least one non-target specific criterion comprises the presence or absence of particular nucleotides at at least three of sequence positions 1 - 19 of the at least one candidate siRNA, wherein any two of more of said particular nucleotides may be the same or different nucleotides.
32. (new) The method according to claim 1, wherein the at least one non-target specific criterion comprises the presence or absence of particular nucleotides at at least four of sequence positions 1 - 19 of the at least one candidate siRNA, wherein any two or more of said particular nucleotides may be the same or different nucleotides.
33. (new) The method according to claim 1, wherein the at least one non-target specific criterion comprises the presence or absence of particular nucleotides at at least five of sequence positions 1 - 19 of the at least one candidate siRNA, wherein any two or more of said particular nucleotides may be same or different nucleotides.
34. (new) The method according to claim 33, wherein said antisense strand is 100% complementary to said target sequence.
35. (new) The method according to claim 1, wherein said rationally designed siRNA has a functionality of at least 70%.
36. (new) The method according to claim 35, wherein said rationally designed siRNA has a functionality of at least 80%.
37. (new) The method according to claim 36, wherein said rationally designed siRNA has a functionality of at least 95%.